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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/581,814	08/22/2007	Patrice Marche	045636-5083	7396

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EXAMINER	
WOOLWINE, SAMUEL C	
ART UNIT	PAPER NUMBER
1637	
MAIL DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/581,814	MARCHE ET AL.	
	Examiner	Art Unit	
	SAMUEL C. WOOLWINE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 January 2011*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *23-35* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *23-35* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *04/29/2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 01/11/2011 has been entered.

The objection to claims 23 and 24 made in the Office action mailed 07/13/2010 (page 3) is withdrawn in view of the amendment.

The rejection of claims 23-36 under 35 USC 112, 2nd paragraph made in the Office action mailed 07/13/2010 is withdrawn in view of the amendment.

The rejections under 35 USC 103 made in the Office action mailed 07/13/2010 are withdrawn in favor of new grounds of rejection set forth below. While the Examiner maintains the previous rejections were proper, Applicant has made the argument that one of skill would not have thought it possible to amplify long fragments of human genomic DNA, able to be seen by gel electrophoresis, using amounts of DNA that could be obtained from an individual. Applicant argues the Barnes reference used bacteriophage lambda DNA as a template (this reference was relied upon to show long fragments of DNA could be amplified and visualized by gel electrophoresis). Applicant makes mathematical calculations based on the size difference between bacteriophage lambda genomic DNA and human genomic DNA and comes to the conclusion that one

would need to use 2-200 mg of human genomic DNA (paragraph spanning pages 12-13 of the response). Therefore, the Examiner has incorporated another reference to rebut this argument.

The objection to the Sequence Listing made in the Office action mailed 07/13/2010 is maintained and reiterated below. Applicant has indicated that a substitute Sequence Listing was supplied with the response (page 8), but it does not appear that a substitute Sequence Listing was supplied. The latest Sequence Listing showing in the file was submitted 04/29/2010, which was the one objected to.

Sequence Compliance

It is noted that the Sequence Listing supplied on 04/29/2010 has been found technically deficient (see entry on 05/06/2010 in Private Pair). An appropriately corrected Sequence Listing is required (see MPEP 2426 and 37 CFR 1.825 for the requirements for amendments to or replacement of Sequence Listings).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation

under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 23-26, 28-30, 33-35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pasqual et al (J Exp Med 196(9):1163-73, Nov 2002, cited on the IDS of 06/05/2006) [as evidenced by Biochemica (No. 3, pp. 4-5, 1995, prior art of record) and Krangel (Nature Immunology 4(7):624-630, July 2003, cited on the IDS of 02/26/2007)] in view of:

1) GenBank GI:21363121 [online] 06/10/2002 [retrieved on 10/26/2009] retrieved from: http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21363121:OLD03:2443007 (prior art of record)

2) Arstila et al (Science 286:958-61, October 1999, cited on the IDS of 02/26/2007)

3) Kolmodin (Methods in Molecular Biology, Vol 192: PCR Cloning Protocols, 2nd edition, Humana Press, pp 37-51 (2002)),

4) Barnes (PNAS 91:2216-2220, March 1994, prior art of record), and

5) Wu et al (US 5,756,701, prior art of record) [this reference included only to meet the limitations of claim 25 and, by dependency, claim 26].

With regard to claims 23 and 24, Pasqual taught a method for the quantitative evaluation of TCRAD gene rearrangement in the mouse. The method comprised extraction of genomic DNA from a biological sample (paragraph spanning pages 1164-

5). The method comprised amplification of multiple different segments of said genomic DNA resulting from different VJ rearrangements (paragraph spanning pages 1164-5: maximum amplicon size was ~5 kb; see also figure 1). The primers used in Pasqual's method comprised "V" primers and "J" primers (paragraph spanning pages 1164-5). Since the V gene RSS flanks the 3' end (i.e. lies downstream) of the V gene and the J gene RSS flanks the 5' end (i.e. lies upstream) of the J gene (see figure 4 of Krangel et al), Pasqual's V primer inherently hybridized upstream of the RSS of the V gene, while the J primer inherently hybridized downstream of the RSS sequence of the J gene.

Pasqual's method used the Expand High Fidelity PCR system (paragraph spanning pages 1164-5). As evidenced by the Biochemica article, the Expand High Fidelity PCR system is a blend of Taq and Pwo polymerases, the latter having proofreading (i.e. correction) activity, the blend substantially improving elongation. Therefore, Pasqual's method inherently met this limitation.

Pasqual's amplification comprised an initial denaturing step (paragraph spanning pages 1164-5: 5 min at 94°C) and cycles of denaturation, hybridization and elongation (at 72°C, for six minutes; paragraph spanning pages 1164-5).

Pasqual's method comprised separation of amplified fragments by electrophoretic migration on a gel (page 1165, first full paragraph: separation by agarose gel electrophoresis).

Pasqual's method also comprised detection of the recombined VJ segments (page 1165, first full paragraph: Southern blot of the gel followed by hybridization with probes).

With regard to claim 25, Pasqual taught using primers "specific" for given V_x and J_y genes (paragraph spanning pages 1164-5). This implicitly teaches selection of primers whose 3'OH ends are complementary only to the regions of interest.

With regard to claim 28, Pasqual performed PCR on purified (i.e. extracted) genomic DNA (paragraph spanning pages 1164-5).

With regard to claims 33 and 34, Pasqual taught T lymphocytes (T cells) from thymus (i.e. thymocytes).

With regard to claims 23 and 24, Pasqual did not perform the method on human genomic DNA obtained from an individual (but rather, mouse genomic DNA) and did not use samples that were either blood or biopsies (the plain meaning of "biopsy" is a sample obtained from a living subject; Pasqual did not state whether the mice from which the thymus samples were taken were living or had been euthanized, although the latter is more probable). Pasqual did not perform the elongation steps of the PCR for 10 minutes. Pasqual performed only the final elongation step for 10 minutes (the claims require "steps", plural). Pasqual performed the other elongation steps for 6 minutes (paragraph spanning pages 1164-5). Pasqual did not teach performing electrophoresis in the presence of a DNA-labeling agent and detecting the amplified segments directly on the gel after excitation in the UV range or other appropriate wavelength.

With regard to claim 25, Pasqual did not teach "systematic analysis of the entire locus concerned, and in particular of the human TCRAD locus, using suitable software". Nor did Pasqual teach elimination of primers forming autodimers or stable hairpins or primers that form hybrids with one another.

With regard to claim 26, Pasqual did not teach primers selected from SEQ ID NOs: 1-21.

With regard to claim 29, Pasqual did not teach incrementing the elongation step by 15-20 seconds per cycle.

With regard to claim 30, Pasqual did not teach pulsed field migration.

With regard to claim 35, Pasqual did not teach amplified fragments greater than 10 kb.

With regard to claims 23 and 24, Arstila taught amplification of human complementary DNA (cDNA, i.e. not genomic DNA) for analysis of T cell receptor αβ diversity (see entire article, e.g. page 958, 2nd column, last paragraph; page 959, paragraph spanning columns 2-3). Arstila obtained T cells from blood and used these T cells to produce the cDNA for amplification (page 958, column 2: "Complementary DNA from 10^8 peripheral blood T cells from a healthy donor...".

With regard to amplifying long fragments from human genomic DNA, Kolmodin taught that 24.2 kb fragments could be amplified from "nanograms quantities" of genomic DNA (page 37, first paragraph). Kolmodin also taught that blends of polymerases having proofreading activity (see claims 23, 24: "correction activity") as well as increases extension time allowed for the amplification of long fragments (page 37, enumerated items 1 and 5). Kolmodin also taught an extension time (elongation) of at least 10 minutes, in particular 12 minutes, and with regard to claim 29, taught incrementing the elongation step by 15 seconds per cycle (page 42, first set of enumerated items, item 4). Kolmodin also taught that the amplified products could be

visualized by agarose gel electrophoresis, in particular pulsed field gel electrophoresis, and stained with ethidium bromide (a DNA-labeling reagent; page 42, second set of enumerated items, items 2-3). Finally, Kolmodin taught that in general, 50-100 ng of human genomic DNA would suffice (page 47, note 20). This is about a million times less than the 2-200 mg Applicant asserts would be required (paragraph spanning pages 12-13 of response). Although Kolmodin taught staining with ethidium bromide, presumably after the electrophoretic separation, she did not teach performing the electrophoretic separation *in the presence of* ethidium bromide.

With regard to claims 23 and 24, Barnes taught the generation by PCR of fragments as long as 35 kb (see title) and the direct visualization of these PCR products in gels run with or later stained by ethidium bromide (a DNA labeling agent; see page 2216, column 2, "Agarose Gel Electrophoresis" and see figure 3c). In particular, Barnes taught pulsed field electrophoresis (*id.*). Note also that Barnes taught a performing the extension step of the PCR at 68°C for 11-24 minutes, which meets the limitation of claims 23 and 24, step b(ii).

With regard to claim 25, Wu taught selection of specific primer pairs and probes for analyzing specific analytes (see title). Wu taught (beginning at column 18, line 50: "PRIMER OPTIMIZATION"):

> An analysis and modification of the original primers was undertaken with the objectives of improving the amplification efficiency of each primer and decreasing the possibility of cross-reactivity among the primer pairs. Several parameters were manipulated in order to minimize physical property differences among the primers. Each primer was modified to approximately the same length, i.e., 19-24 bp. Primer oligonucleotides of this length result in greater specificity in the amplification reaction while shorter primers may result in the amplification of non-specific products. Because the efficiency of the primer pairs is also effected by the presence of hairpin loops and dimers, OLIGO 5.0 software (NBI, Plymouth, Minn., USA) was used to analyze potential primers. If hairpin loops or dimers

were found, the primer sequence was modified to remove them or to, at least, diminish the effect.

With regard to claims 26 and 35, GenBank GI:21363121 disclosed the sequence of the human T cell receptor alpha/delta locus on chromosome 14. At least SEQ ID NOs: 1 and 11 (and presumably all the other SEQ ID NOs from 1-21) are found within the sequence disclosed by GenBank GI:21363121:

```
GenBank         128261  GGTCGTTTTTCTTCATTCCTTAGTCG  128286
                        |||||||||||||||||||||||||
SEQ ID NO:1     1       GGTCGTTTTTCTTCATTCCTTAGTCG  26


GenBank         989703  GTAAGTTTGAAGGGAGTGGGGGAAG  989727
                        |||||||||||||||||||||||||
SEQ ID NO:11    25      GTAAGTTTGAAGGGAGTGGGGGAAG  1
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With regard to claim 35, based upon the known sequence of the human TCRAD locus disclosed by GenBank, one of skill in the art would have expected that carrying out PCR similar to that disclosed by Pasqual would potentially generate amplicons greater than 10 kb, depending on the types of VJ recombination that took place in the individual human subject.

It would have been *prima facie* obvious to one or ordinary skill in the art at the time the invention was made to apply the method taught by Pasqual for analyzing T cell receptor diversity to humans, using samples such as T cells obtained from blood, as taught by Arstila. One would have been motivated to do this because it was clearly of interest to those in the field to assess human T cell receptor diversity, as shown by the disclosure of Arstila. Pasqual stated (abstract): "Knowledge of the complete nucleotide sequence of the mouse TCRAD locus allows an accurate determination V-J rearrangement status." Likewise, one of ordinary skill would have reasoned that

knowledge of the complete sequence of the human TCRAD locus (as disclosed by GenBank GI:21363121) would allow similar analysis of human T cell receptor V-J rearrangements. Hence one would have been motivated to use the known human sequence to select appropriate primers for the various V and J segments, just as Pasqual did for the mouse.

Using primer design software, and choosing primers that were specific, free of secondary structure, and that were unlikely to form dimers (either autodimers or heterodimers with other primers being used) were well-known principles in the art of designing primers, as shown by the disclosure of Wu.

Pasqual's method offered an advantage over the earlier method of Arstila, in that the latter artisan's method was based on amplification of cDNA (i.e. analysis at the transcriptional level) and for only specific V and J genes. Pasqual discussed these shortcomings (page 1164, column 2, 1st and 2nd paragraphs): "Finally to date, available informations encompass essentially either analysis at the transcriptional level or gene analysis for only a few V families, thus precluding a general synthetic overview of gene rearrangements. In this report, to eliminate the biases due to transcriptional regulation…we have used a sensitive multiplex PCR assay at the genomic DNA level."

Hence one would have been motivated to supplant the approach used by Arstila with the technique of Pasqual to study human TCR diversity, to avoid bias caused by transcriptional regulation and provide a general synthetic overview of gene rearrangements.

It would also have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made in view of the disclosures of Kolmodin and Barnes to use an at least 10 minute extension step, to increment the extension step by 15 seconds per cycle, and to use pulsed-field electrophoresis to separate the amplified fragments; one of ordinary skill in the art would have been aware, based on the sequence of the human TCRAD locus disclosed in GenBank, of the expected lengths of the amplification products, and would have known that pulsed field gel electrophoresis was a better way to resolve such long fragments compared to standard electrophoresis. It would also have been obvious to include ethidium bromide in the gels during electrophoretic separation or to stain the gels afterward (as taught by Barnes), and to visualize the products directly on the gel in order to dispense with the need to perform Southern blot, which would have thus saved time and expense and avoided the use of radioactive materials.

Claim 27 is rejected under 35 U.S.C. 103(a) as being unpatentable over Pasqual et al (J Exp Med 196(9):1163-73, Nov 2002, cited on the IDS of 06/05/2006) [as evidenced by Biochemica (No. 3, pp. 4-5, 1995, prior art of record) and Krangel (Nature Immunology 4(7):624-630, July 2003, cited on the IDS of 02/26/2007)] in view of:

1) GenBank GI:21363121 [online] 06/10/2002 [retrieved on 10/26/2009] retrieved from: http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21363121:OLD03:2443007 (prior art of record)

2) Arstila et al (Science 286:958-61, October 1999, cited on the IDS of 02/26/2007)

3) Kolmodin (Methods in Molecular Biology, Vol 192: PCR Cloning Protocols, 2nd edition, Humana Press, pp 37-51 (2002)),

4) Barnes (PNAS 91:2216-2220, March 1994, prior art of record), and

5) Wu et al (US 5,756,701, prior art of record)

as applied to claims 23-26, 28-30, 33-35 above, and further in view of GenBank GI:21536269 [online] June 21, 2002 [retrieved on October 27, 2009] retrieved from http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21536269:OLD03:2443019 (prior art of record).

The teachings of Pasqual, GenBank GI:21363121, Arstila, Kolmodin, Barnes and Wu have been discussed.

With regard to claim 27, while Pasqual did not analyze V, D or J segments of the TCR β chains, Arstila did analyze TCR β chain gene rearrangement by PCR with primers to specific V_β and J_β segments (e.g. page 958, 2nd column, last paragraph).

GenBank GI: 21536269 disclosed the complete sequence of the human TCR β locus.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the method taught by Pasqual for analyzing T cell receptor diversity to human TCR β chain gene rearrangements. Those of in the field were interested in such analyses as shown by the disclosure of Arstila. Pasqual stated (abstract): "Knowledge of the complete nucleotide sequence of the mouse TCRAD

locus allows an accurate determination V-J rearrangement status." Likewise, one of ordinary skill would have reasoned that knowledge of the complete sequence of the human TCR β locus (as disclosed by GenBank GI:21536269) would allow similar analysis of human T cell receptor β chain V(D)J rearrangements.

Pasqual's method offered an advantage over the earlier method of Arstila, in that the latter artisan's method was based on amplification of cDNA (i.e. analysis at the transcriptional level) and for only specific V and J genes. Pasqual discussed these shortcomings (page 1164, column 2, 1st and 2nd paragraphs): "Finally to date, available informations encompass essentially either analysis at the transcriptional level or gene analysis for only a few V families, thus precluding a general synthetic overview of gene rearrangements. In this report, to eliminate the biases due to transcriptional regulation…we have used a sensitive multiplex PCR assay at the genomic DNA level." Hence one would have been motivated to supplant the approach used by Arstila with the technique of Pasqual to study human TCR diversity, to avoid bias caused by transcriptional regulation and provide a general synthetic overview of gene rearrangements.

Claims 31 and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Pasqual et al (J Exp Med 196(9):1163-73, Nov 2002, cited on the IDS of 06/05/2006) [as evidenced by Biochemica (No. 3, pp. 4-5, 1995, prior art of record) and Krangel (Nature Immunology 4(7):624-630, July 2003, cited on the IDS of 02/26/2007)] in view of:

1) GenBank GI:21363121 [online] 06/10/2002 [retrieved on 10/26/2009] retrieved from: http://www.ncbi.nlm.nih.gov/sviewer/viewer.fcgi?21363121:OLD03:2443007 (prior art of record)

2) Arstila et al (Science 286:958-61, October 1999, cited on the IDS of 02/26/2007)

3) Kolmodin (Methods in Molecular Biology, Vol 192: PCR Cloning Protocols, 2nd edition, Humana Press, pp 37-51 (2002)),

4) Barnes (PNAS 91:2216-2220, March 1994, prior art of record), and

5) Wu et al (US 5,756,701, prior art of record)

as applied to claims 23-26, 28-30, 33-35 above, and further in view of Dau et al (US 6,087,096, prior art of record).

The teachings of Pasqual, GenBank GI:21363121, Arstila, Kolmodin, Barnes and Wu have been discussed. These references did not teach analyzing T-cell receptor profiles (repertoire) for the purpose of monitoring pathology or a response to treatment in a subject, or comparing the profile of a subject to a "standard immune repertoire".

Dau taught (paragraph spanning pages 13-14):

> The ability to characterize an individual's T cell repertoire has practical applications for monitoring treatments for innumerable disorders, because the efficacy of many treatments lies in their ability to modulate (to potentiate or to suppress) an immune response. For example, when an individual is afflicted with many disorders (e.g., neoplastic disorders, chronic infection), it is desirable to provide a treatment designed to potentiate the individual's own immune response to the disorder, to suppress or overcome the disorder (i.e., it is desirable to provide an immunoproliferative treatment). A method for characterizing an individual's T cell repertoire which detects a T cell immunoproliferative response to a treatment is useful for monitoring the efficacy of such a treatment. A first characterization of the T cell repertoire as it exists prior to the treatment is compared to a second characterization of the T cell repertoire during or after the treatment to detect the presence or absence of a T cell immunoproliferative response to the treatment. Characterizations may be repeated to continue to monitor the treatment and/or to monitor for a relapse of the disorder between treatments.

Dau also taught comparing the T cell repertoire in a subject to that of a healthy human subject (paragraph spanning columns 3-4).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the method for analyzing human TCR profiles suggested by the combined teachings of Pasqual, GenBank GI:21363121, Arstila, Kolmodin, Barnes and Wu for the purpose of monitoring response to treatment (which also represents "phases of pathology") in an individual, since it was known in the prior art as taught by Dau to use TCR profiles as an indication of response to treatment by comparing pre- and post-treatment TCR profiles, as well as to use TCR profiles as an indication of the presence of disease by comparing an individual's TCR profile to that of a healthy individual (i.e. standard immune repertoire).

Response to Arguments

Applicant's arguments filed 01/11/2011 have been fully considered but they are not persuasive. The Examiner will first address the declaration proffered by Dr. Pasqual. The assertions in the declaration are basically two: (1) that the more genomic DNA is included in a PCR reaction, the less efficient the amplification becomes and (2) that in order to obtain a copy number for human genomic DNA equivalent to the copy number of phage DNA used by Barns, far more than 2 µg of human genomic DNA would be required. Both of these arguments are premised on the position that great amounts of DNA would be required to amplify such large fragments from human genomic DNA. These arguments are not persuasive for several reasons. First and foremost, the newly

cited reference, Kolmodin, clearly states that only nanogram quantities of human genomic DNA are needed to produce long PCR products (specifically 50-100 ng; see rejection above). As further evidence, one of the references cited by Kolmodin (Cheng et al, PNAS 91:5695-5699 (1994), cited reference 4, provided herewith) obtained PCR products of up to 22 kb using only 37 nanograms of total human genomic DNA (see figure 5). Therefore, the argument that one of skill in the art would not have believed it possible to use PCR to amplify fragments greater than 10 kb is not persuasive. Kolmodin clearly taught 50-100 ng as sufficient.

Furthermore, whether there is a point at which the amount of genomic DNA in a PCR reaction becomes inhibitory is irrelevant since the prior art shows that large PCR products could be obtained with very low amounts of genomic DNA.

As far as the experiment conducted in the declaration, it is noted that (1) the PCR parameters used and the target size being amplified do not reflect those in the claims or the references, and (2) it is not clear how each tube receiving 25 ng of murine gDNA controls for viscosity, since the total amount of gDNA (i.e. murine + fish) still varies from tube to tube. However, it is respectfully submitted that simply showing an experimental PCR that did not produce a product does not alter the fact that successful amplification of fragments greater than 10 kb using only nanograms amounts of DNA had already been achieved in the prior art 10 years prior to Applicant's filing (referring to the rebuttal reference Cheng et al).

Turning now to Applicant's other arguments (beginning at page 11 of the response), it is respectfully submitted that Pasqual's use of Southern blot as a means of

detecting the amplified fragments does not constitute a teaching away from using ethidium bromide as a means of detection. The Examiner does not argue whether the amount of amplified material obtained by Pasqual was sufficient for detection by ethidium bromide, but rather that the prior art (Barnes, Kolmodin) demonstrates that such amounts of amplified PCR product *could* be obtained using the knowledge disclosed in the prior art, particularly Kolmodin, who provided adequate guidance for optimizing PCR conditions.

All of Applicant's remaining arguments follow a similar path, asserting that one would have needed to use so much human genomic DNA to amplify long PCR products, that the amount of genomic DNA in the reaction itself would have become inhibitory to the PCR reaction. For the reasons discussed above, and for the reasons set forth in the rejection based on Kolmodin, the Examiner is not persuaded by such arguments.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL C. WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/
Primary Examiner